PATENT
Attorney Docket No. **AMBER-06311**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Kenneth J. Rothschild *et al.*		
Serial No.:	09/813,197	Group No.:	1636
Filed:	3/20/01	Examiner:	
Entitled:	**Methods For The Detection, Analysis And Isolation Of Nascent Proteins**		

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(I)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: January 2, 2002 By: _____
Christopher J. Collins

Sir or Madam:

Enclosed please find an Information Disclosure Statement and Form PTO-1449, including copies of the references contained thereon, for filing in the U.S. Patent and Trademark Office.

The Commissioner is hereby authorized to charge any additional fee or credit overpayment to our Deposit Account No. **08-1290**. **An originally executed duplicate of this transmittal is enclosed for this purpose.**

Dated: January 2, 2002

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Sir or Madam:

The citations listed below, copies attached, may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97. The Examiner is requested to make these citations of official record in this application.

The following printed publications are referred to in the body of the specification:

- U.S. Pat. No. 4,683,195 to Mullis *et al.*;
- U.S. Pat. No. 4,774,339 to Haugland *et al.*;
- U.S. Pat. No. 5,069,769 to Fujimiya *et al.*;
- U.S. Pat. No. 5,091,328 to Miller;
- U.S. Pat. No. 5,137,609 to Manian *et al.*;
- U.S. Pat. No. 5,187,288 to Kang *et al.*;
- U.S. Pat. No. 5,190,632 to Fujimiya *et al.*;
- U.S. Pat. No. 5,248,782 to Haugland *et al.*;

- U.S. Pat. No. 5,274,113 to Kang *et al.*;
- U.S. Pat. No. 5,433,896 to Kang *et al.*;
- U.S. Pat. No. 5,451,663 to Kang *et al.*;
- U.S. Pat. No. 5,643,722 to Rothschild *et al.*;
- U.S. Pat. No. 5,783,397 to Hughes *et al.*;
- PCT WO90/05785 to Schultz;
- U.S. Pat. No. 565,451[1];
- Allen *et al.*, *Gel Electrophoresis and Isoelectric Focusing of Proteins*, Walter de Gruyter, New York 1984, pp.17-62;
- *Antibodies: A Laboratory Manual* (E. Harlow and D. Lane, editors, Cold Spring Harbor Laboratory Press, 1988) pp.53,72-73;
- Bain *et al.*, "Site-Specific Incorporation of Nonnatural Residues during In Vitro Protein Biosynthesis with Semisynthetic Aminoacyl-tRNAs," *Biochemistry* 30:5411-21 (1991);
- Bruce and Uhlenbeck, "Specific Interaction of Anticodon Loop Residues with Yeast Phenylalanyl-tRNA Synthetase," *Biochemistry* 21:3921-3926 (1982);
- *Current Protocols in Molecular Biology* (F.M. Ausubel et al. editors, Wiley Interscience, 1993), 10-16,10-77;
- Da Poian, A. T., *et al.*, "Kinetics of intracellular viral disassembly and processing probed by Bodily fluorescence dequenching," *J Virol Methods* 70(1), 45-58 (1998);
- Doty *et al.*, "Strand Separation and Specific Recombination in Deoxyribonucleic Acids: Physical Chemicals Studies," *Proc. Natl. Acad. Sci. USA* 46:461 (1960);
- DiCesare et al., "A High-Sensitivity Electrochemiluminescence-Based Detection System for Automated PCR Product Quantitation," *BioTechniques* 15:152-59 (1993);
- Felgner *et al.*, "Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure," *Proc. Natl. Acad. Sci. USA* 84:7413-17 (1987);

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[1] This patent was incorrectly cited in the application as filed. We are trying to determine the correct patent number.

Happ *et al.*, "New Approach to the Synthesis of 2'(3')-*O*-Aminoacyl Oligoribonucleotides," *J. Org. Chem.* 52:5387-91 (1987);
Heckler *et al.*, "Preparation of 2'(3')-O-Acyl-pCpA Derivatives as Substrates for T4 RNA Ligase-Mediated "Chemical Aminoacylation"," *Tetrahedron* 40:87-94 (1984);

- Heckler *et al.*, "T4 RNA Ligase Mediated Preparation of Novel "Chemically Misacylated" tRNA ^{Phe}s," *Biochemistry* 23:1468-73 (1984);
- Hemmila, I.A., Chemical Analysis "Applications of Fluorescence in Immunoassays", (Wiley&Sons 1991) pp.138-159;
- Hudson, "Methodological Implications of Simultaneous Solid-Phases Peptide Synthesis. 1. Comparison of Different Coupling Procedures," *J. Org. Chem.* 53:617-624 (1988);
- Krieg *et al.*, "Photocrosslinking of the signal sequence of nascent preprolactin to the 54-kilodalton polypeptide of the signal recognition particle," *Proc. Natl. Acad. Sci. USA* 83:8604-08 (1986);
- Keller, R. C., *et al.*, "Characterization of the Resonance Energy Transfer Couple Coumarin-Bodily and its Possible Applications in Protein-Lipid Research," *Biochem Biophys Res Commun* 207(2), 508-14 (1995);
- Kim, D., and Choi, C., "A Semicontinuous Prokaryotic Coupled Transcription/Translation System Using a Dialysis Membrane," *Biotechnol Prog* 12, 645-649 (1996) ;
- Kopp *et al.*, "Chemical Amplification: Continuous Flow PCR on a Chip," *Science* 280:1046 (1998);
- Kozak, "Point Mutations Define a Sequence Flanking the AUG Initiator Codon that Modulates Translation by Eukaryotic Ribosomes," *Cell* 44:283-292 (1986);
- Kudlicki, W.*et al.*, "Chaperone-dependent Folding and Activation of Ribosome-bound Nascent Rhodanese," *J Mol Biol* 244(3), 319-31 (1994);
- Laemmli, U. K., "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4," *Nature* 227:680-685 (1970);

- Marmur and Lane, "Strand Separation and Specific Recombination in Deoxyribonucleic Acids: Biological Studies," *Proc. Natl. Acad. Sci. USA* 46:453-461 (1960);
- *Molecular Cell Biology* (J. Darnell et al. editors, Scientific American Books, N.Y., N.Y. 1991) pp.119-132;
- Neu and Heppel, "Nucleotide Sequence Analysis of Polyribonucleotide by Means of Periodate Oxidation Followed by Cleavage with an Amine," *J. Biol. Chem.* 239:2927-34 (1964);
- Noren *et al.*, "A General Method for Site-Specific Incorporation of Unnatural Amino Acids into Proteins," *Science* 244:182-188 (1989);
- Odom, O. W, *et al.*, "In vitro engineering using acyl-derivatized tRNA," In Protein synthesis: Methods and Protocols, PP.93-103, (Humana Press, Totowa, NJ.);
- Patchornik *et al.*,"Photosensitive Protecting Groups," *J. Am. Chem. Soc.* 92:6333-35 (1970);
- Pavlopoulos, *et al.*, "Laser action from a tetramethylpyrromethene-BF.sub.2 complex," *APP. OPTICS* 27:4998-4999 (1988);
- Pfahler *et al.*, Sensors and Actuators, A21-A23 , pp. 431-434 (1990)[2];
- Pillai, "Photoremovable Protecting Groups in Organic Synthesis," *Synthesis* 1-26 (1980);
- Powell *et al.*, "Molecular Diagnosis of Familial Adenomatous Polyposis," *N. Engl. J. Med.* 329:1982-87 (1993);
- Pratt, "Coupled Transcription-Translation in Prokaryotic Cell-Free System," (*Transcription and Translation*, B.D. Hames and S.J. Higgins, Editors, p. 179-209, IRL Press, Oxford, 1984);
- Promega Technical Bulletin No. 182; tRNA$^{nscend™}$: Non-radioactive Translation Detection System, Sept. 1993;
- Reis, R. C., *et al.*, "A novel methodology for the investigation of intracellular proteolytic processing in intact cells," *Eur J Cell Biol* 75(2), 192-7 (1998);

[2] We have been unable to obtain this reference, but if the examiner requests a copy we will again seek to obtain it.

Rowan and Bodmer, "Introduction of a *myc* Reporter Taq to Improve the Quality of Mutation Detection Using the Protein Truncation Test," *Human Mutation* 9:172-176 (1997);

- Sampson and Uhlenbeck, "Biochemical and physical characterization of an unmodified yeast phenylalanine transfer RNA transcribed *in vitro*," *Proc. Natl. Acad. Sci. USA* 85:1033-37 (1988);
- Seong and RajBhandary, "*Escherichia coli* formylmethione tRNA: Mutations in ^{GGG} sequence conserved in anticodon stem of initiator tRNAs affect initiation of protein synthesis and conformation of anticodon loop," *Proc. Natl. Acad. Sci. USA* 84:334-338 (1987);
- Spirin *et al.*, "A Continuous Cell-Free Translation System Capable of Producing Polypeptides in High Yield," *Sci.* 242:1162-64 (1988);
- Stephen, "High-Resolution Preparative SDS-Polyacrylamide Gel Electrophoresis: Fluorescent Visualization and Electrophoretic Elution-Concentration of Protein Bands," *Anal. Biochem.* 65:369-79 (1975);
- Treibs & Kreuzer, "Difluorboryl-komplexe von di- und tripyrrylmethenen," *Liebigs Ann. Chem.* 718:208-223 (1968);
- Turcatti *et al.*, "Probing the Structure and Function of the Tachykinin Neurokinin-2 Receptor through Biosynthetic Incorporation of Fluorescent Amino Acids at Specific Sites," *J Biol Chem* 271(33), 19991-8 (1996);
- Van Lintel *et al.*, *Sensors and Actuators* 15:153-167 (1988)[3];
- Varshney U. and RajBhandary UL, "Initiation of protein synthesis from a termination codon," Proc Natl Acad Sci U S A 87(4):1586-90 (1990);
- Varshney *et al.*, "Direct Analysis of Aminoacylation Levels of tRNAa in Vivo," *J. Biol. Chem.* 266: 24712-24718 (1991);
- Yao S *et al.*, "SDS capillary gel electrophoresis of proteins in microfabricated channels," *PNAS* 96:5372-5377 (1999);
- Vecesey-Semjen *et al.*, "The Staphylococcal α-Toxin Pore Has a Flexible Conformation," *Biochemistry* 38 4296-4302 (1999);

[3] We have been unable to obtain this reference, but if the examiner requests a copy we will again seek to obtain it.

Vos de Waal *et al.* (1977)[4];

Walker, B. *et al.*,"Functional Expression of the α-Hemolysin of Staphylococcus aureus in Intact Escherichia coli and in Cell Lysates," *J. Biol. Chem.* 267:10902-10909 (1992); and

- Wories *et al.*, "A novel water-soluble fluorescent probe: Synthesis, luminescence and biological properties of the sodium salt of the 4-sulfonato-3,3', 5'5-tetramethyl-2,2'-pyrromethen-1,1'-BF.sub.2 complex," *Recl. Trav. Chim. PAYSBAS* 104, 288 (1985)[5];

Applicants have become aware of the following printed publications which may be material to the examination of this application:

- U.S. Pat. No. 4,675,285 to Clark *et al.*, provides a method for identification of clones expressing the desired protein from the cDNA libraries. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- U.S. Pat. No. 5,709,998 to Kinzler *et al.*, describes APC gene, its mutations and mutations linked condition (familial adenomatous polyposis, FAP). Primers, and method for mutation detection using protein truncation test with radioactive readout is also provided. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- U.S. Pat. No. 5,861,494 to Soppet *et al.*, describes APC gene, its mutations and mutations linked condition (familial adenomatous polyposis, FAP). Primers, and method for mutation detection using protein truncation test with radioactive readout is also provided. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

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[4] We have been unable to obtain this reference, but if the examiner requests a copy we will again seek to obtain it.

[5] We have been unable to obtain this reference, but if the examiner requests a copy we will again seek to obtain it.

U.S. Pat. No. 5,622,829 to King *et al.*, disclose the sequences of breast and ovarian cancer susceptibility genes (BRCA1). Hybridization-based methods are also described for the detection of specific mutations. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- U.S. Pat. No. 5,693,473 to Shattuck-Eidens *et al.*disclose the sequences of breast and ovarian cancer susceptibility genes (BRCA1). Hybridization-based methods are also described for the detection of specific mutations. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- U.S. Pat. No. 5,760,207 to Kinzler *et al.*, describes APC gene, its mutations and mutations linked condition (familial adenomatous polyposis, FAP). Primers, and method for mutation detection using protein truncation test with radioactive readout is also provided. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- U.S. Pat. No. 5,879,890 to Laken *et al.*, describes APC gene, its mutations and mutations linked condition (familial adenomatous polyposis, FAP). Primers, and method for mutation detection using protein truncation test with radioactive readout is also provided. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Czworkowski *et al.*, "Fluorescence Study of the Topology of Messenger RNA Bound to the 30S Ribosomal Subunit of *Escherichia coli*," *Biochemistry* 30:4821-4830 (1991), describes the interaction of fluorescently labeled RNAs (25-36 nucleotides in length) with the fluorescently labeled 30S subunit of Escherichia coli studied by using fluorescence spectroscopic techniques. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Hardesty *et al.*, "Ribosome function determined by fluorescence," *Biochimie* 74:391-401 (1992). A review on ribosome function and the position and growth of the nasent peptide using aminoacyl-tRNAs comprising a coumarin tag. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Kudlicki et al., "Chaperone-dependent Folding and Activation of Ribosome-bound Nascent Rhodanese," *J. Mol. Biol.* 244:319-331 (1994), Coumarin labeled rhodanese was synthesized by coupled transcription/translation in a cell-free Escherichia coli system. The influence of chaperones on the release of nascent rhodanese from ribosome was studied by fluorescence spectroscopy. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Picking *et al.*, "The use of synthetic tRNA as probes for examining nascent peptides on *Escherichia coli* ribosomes," *Biochimie* 73:1101-1107 (1991), the cell free synthesis of N-acetyl or N-acyl coumarin labeled polycysteine and polyserine were carried out on Escherichia coli ribosomes using N-acyl coumarin derivatives of either Ser-tRNA or Phe-tRNA. The properties of the resulting nascent peptides were studied by fluorescence spectroscopy and compared to those of nascent polyphenylalanine chains synthesized under similar conditions. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Picking *et al.*, "Evidence for RNA in the Peptidyl Transferase Center of Escherichia coli Ribosomes as Indicated by Fluorescence," *Biochemistry* 31:12565-12570 (1992), The interaction of coumarin labeled (tRNA(phe)) [either the amino acid or the 5' end] with ribosomes was studied using fluorescence spectroscopy. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Picking *et al.*, "The Conformation of Nascent Polylysine and Polyphenylalanine Peptides on Ribosomes," *J. of Biological Chemistry* 266:1534-1542 (1991), describes the behavior of fluorescently labeled polylysine and polyphenylalanine during their in vitro synthesis on E. coli ribosomes was studied. The position and conformation of the nascent peptide were monitored by fluorescence techniques. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Picking *et al.*, "Fluorescence Characterization of the Environment Encountered by Nascent Polyalanine and Polyserine as They Exit Escherichia coli Ribosomes during Translation," *Biochemistry* 31:2368-2375 (1992). A coumarin probe placed at the alpha-amino group of a synthetic elongator alanyl-tRNA or a synthetic initiator alanyl-tRNA or at the epsilon-amino group of natural lysyl-tRNA, and each was used to nonenzymatically initiate peptide synthesis. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Picking *et al.*, "A synthetic alanyl-initiator tRNA with initiator tRNA properties as determined by fluorescence measurements: Comparison to a synthetic alanyl-elongator tRNA," *Nucleic Acids Research* 19:5749-5754 (1991). A derivative of coumarin [3-(4-maleimidophenyl)-7-diethyl-amino-4-methylcoumarin] was covalently attached to the alpha amino group of alanine of the two synthetic Ala-tRNA species. The fluorescence spectra, quantum yield and anisotropy for the two Ala-tRNA derivatives were studied after they were bound to 70S ribosomes. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Ma *et al.*, "In Vitro Protein Engineering Using Synthetic $tRNA^{Ala}$ with Different Anticodons," *Biochemistry* 32:7939-7945 (1993), describes the use of synthetic tRNA for in vitro protein engineering was tested in a coupled transcription/translation system prepared from *Escherichia coli.* The results

indicate that all four synthetic tRNAs were functionally active in the synthesis of full-length, enzymatically active dihydrofolate reductase protein. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

Odom *et al.*, "Movement of tRNA but Not the Nascent Peptide during Peptide Bond Formation on Ribosomes," *Biochemistry* 29:10734-10744 (1990), The interaction of fluorescently [(3-(4-maleimidophenyl)-7-diethyl-amino-4-methylcoumarin) or (5-[[2-[(iodoacetyl)amino]ethyl]amino]naphthalene-1-sulphonic acid)] labeled (Phe) tRNA at the 5'-end with ribosome as well as with nascent polypeptide was investigated using nonradiative energy transfer. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- U.S. Patent No. 5,614,386 to Metzker *et al.*, describes dyes for use in labeling DNA primers for improved DNA sequencing techniques. The publication does not disclose the misaminoacylation of tRNA with a modified amino acid or other molecule. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- European Pat. No. 0234799A2 to Kurzchalia *et al.*, describes methods for the detection and isolation of protein utilizing the incorporation of photoaffinity reagents and biotin or other haptens into nascent peptides. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Crowley *et al.*, "The signal sequence moves through a ribosomal tunnel into a noncytoplasmic aqueous environment at the ER membrane early in translocation," *Cell* 73:1101-1115 (1993). This reference examines the logistics of nascent peptide production by utilizing εNBD-Lys-tRNA analogs to examine the environment of a nascent peptide chain as it moved through the ribosome

and into the ER membrane. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

Karolin *et al.*, "Fluorescence and Absorption Spectroscopic Properties of Dipyrromethene boron Difluoride (BODIPY) Derivatives in Liquids, Lipid Membranes, and Proteins," *J. Am. Chem. Soc.* 116:7801-7806 (1994) describes the use of a fluorescent dye, (BODIPY) to label a modified plasminogen activator inhibitor protein after the protein had been isolated. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Hardesty *et al.*, "Extension and Folding of Nascent Peptides on Ribosomes." The Translational Apparatus, Nierhaus *et al.* ed: New York and London; Plenum Press. p.347-358 (1993) describes peptide folding experiments where amino-tRNAs were modified after aminoacylation. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Johnson *et al.*, "Protein Synthesis and Secretion as seen by the Nascent Protein Chain," The Translational Apparatus, Nierhaus *et al.* ed: New York and London; Plenum Press. p. 359-370 (1993) discloses peptide synthesis experiments where Lys-tRNA was modified after aminoacylation. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Shore *et al.*, "A Fluorescent Probe Capable of Incorporation into Nascent Polypeptide Chains," 1986 *Federation Proceedings* 45, 1566 Abstract, discloses the attachment of a fluorescent moiety (N^6-fluoresceinthioacetyl) onto a tRNA aminoacylated with lysine wherein the lysine has been modified by reaction with N-bromoacetoxysuccinimide. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Shore *et al.*, "Accessibility of AA-tRNA and Nascent Chain During Protein Synthesis," 1988 *FASEB* Journal 2, A1045 Abstract, discloses the attachment of a fluorescent moiety (N^6-fluoresceinthioacetyl) onto a tRNA aminoacylated with lysine thereby producing N^6-fluoresceinthioacetyl-lys-tRNA. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Bain *et al.*, "Site-Specific Incorporation of Non-Natural Residues into Peptides: Effect of Residue Structure on Suppression and Translation Efficiencies," *Tetrahedron* 47:2389-2400 (1991), discloses methods for the production of a series of 12 semi-synthetic acylated suppressor tRNAs. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Johnson *et al.*, "N-Acetyllysine Transfer Ribonucleic Acid: A Biologically Active Analogue of Aminoacyl Transfer Ribonucleic Acids," *Biochemistry* 15:569-575 (1976), discloses methods for the production of N^6-Acetyl-Lys-tRNA. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Johnson, "Chemically Modified Aminoacyl-tRNA as a Probe of Ribosome Structure: the Synthesis and in vitro Activity of ε-N-acetyl-Lys-tRNA," 1973 Thesis Excerpts, University of Oregon, Eugene, Oregon, discloses several methods of producing fluorescently labeled (N-methylanthranilic acid) Lys-tRNA. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Shore, "The Use of Fluorescent-Labeled Amino Acids to Examine the Environment of Ribosome-Bound Nascent Polypeptide Chains," 1991 Dissertation, University of Oklahoma, Norman Oklahoma, discloses the synthesis of fluorescent analogs of Lys-tRNA including N^6-fluoresceinthioacetyl-Lys-tRNA and N^6-6-(7-nitrobenz-2-oxa-1,3-diazol-4-

yl)aminohexanoyl-Lys-tRNA. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Kramer *et al.*, "In Vitro engineering using synthtic tRNAs with altered anticodons including four-nucleotide anticodons," *Methods Mol Biol.* 77:105-16 (1998) discloses methods for the synthesis of tRNAs with altered anticodons. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Odem *et al.*, "In Vitro engineering using acylderivatized tRNAs," *Methods Mol Biol.* 77:93-103 (1998) discloses a method for the preparation and use of a coumarin derivative of Met-$tRNA_f$. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

Applicants have become aware of the following printed 1999 publications:

- Karginov *et al.*, "Facile characteriation of translation initiation via nonsense codon suppression," *Nucleic Acids Res.* 27:3283-90 (1999) discloses a method for the misacetylation of a tRNA with 3-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-2,3-diaminopropionic acid. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Kramer *et al.*, "N-terminal and C-terminal modifications affect of in vitro synthesized proteins," *Int J Biochem Cell Biol.* 31:231-41 (1999) discloses the effect of certain N-terminal and C-terminal modifications on protein synthesis. This publication modifies a N-acly-Met-$tRNA_f$ with coumarin after acetylation. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.
- Tsalkova *et al.*, "The effect of a hydrophobic N-terminal probe on translational pausing of chloramphenicol acetyl transferase and rhodanese," *J Mol Biol.*

286:71-81 (1999) discloses the effect of hydrophobic residues at the N-terminus in regards to protein synthesis. N-acetyl-S-coumarin-Met-$tRNA_f$ was used to generate the hydrophobic residues. This publication does not disclose a method to produce a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

- Nemoto *et al.*, "Fluorescence labeling of the C-terminus of proteins with a puromycin analogue in cell-free translation systems," *FEBS Letters*. 462:43-46 (1999) discloses a method using puromycin analogues with a fluorescent moiety to label the C-terminus of nascent peptides. This publication does not disclose a charged tRNA/marker conjugate made from a charged tRNA molecule and a coumarin marker which has a sulfonated-NHS leaving group.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

Dated: January 2, 2002

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Registration No. 32,837

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